

- 42 -

**CLAIMS**

1. An attenuated *Actinobacillus pleuropneumoniae* bacterium.
- 5 2. An attenuated *Actinobacillus pleuropneumoniae* bacterium having a mutation in a gene required for bacterial virulence.
3. An attenuated *Actinobacillus pleuropneumoniae* bacterium having a mutation  
10 in a gene which comprises a nucleotide sequence selected from the group consisting of SEQ ID NO.:1-56.
4. An attenuated *Actinobacillus pleuropneumoniae* bacterium having a plurality of mutations, occurring within a single gene or within different genes.
- 15 5. A composition containing an attenuated *A. pleuropneumoniae* bacterium according to any previous claim.
6. A composition according to Claim 5, comprising a plurality of different  
20 attenuated *A. pleuropneumoniae* bacteria, having different mutations in the same virulence gene and/or bacteria having similar, or different, mutations in two or more different genes.
7. A composition according to any one of Claims 5 or 6, further comprising one  
25 or more pharmacologically acceptable adjuvants, diluents, carriers, vehicles and/or excipients.
8. A composition according to any of Claims 5 -7, which is packaged in a form  
30 suitable for administration by intravenous, intradermal, intramuscular, intramammary, intraperitoneal and/or subcutaneous injection, and/or by oral, sublingual, nasal, anal or vaginal delivery.

- 43 -

9. A composition according to any of Claims 5 -8, which is selected from the group consisting of an immunogenic composition, a therapeutic (e.g. prophylactic) composition and a vaccine.
- 5 10. A composition according to Claim 9, wherein the composition is a vaccine whose administration to an animal confers a degree of cross-protection.
11. Use of an attenuated *A. pleuropneumoniae* bacterium according to any of Claims 1-4 in the manufacture of a medicament for preventing or alleviating  
10 an infection of an animal with *A. pleuropneumoniae*.
12. Use of an attenuated *A. pleuropneumoniae* bacterium according to any of Claims 1-4 in the manufacture of a medicament for preventing or alleviating symptoms associated with *A. pleuropneumoniae* infection, such as the  
15 prophylactic protection of swine against porcine pleuropneumonia.
13. An isolated polynucleotide encoding a gene product which is naturally involved in (e.g. required for) the virulence of *A. pleuropneumoniae*.
- 20 14. An isolated polynucleotide encoding a gene product which is not naturally found in *A. pleuropneumoniae*, but whose expression therein is capable of modulating (e.g. of decreasing) the virulence of that bacterium.
15. An isolated polynucleotide which is not naturally found in *A. pleuropneumoniae* but which is capable of modulating the virulence of that  
25 bacterium by its direct interaction with *A. pleuropneumoniae* virulence genes or gene products.
16. An isolated polynucleotide comprising: (a) a nucleotide sequence selected  
30 from the group consisting of SEQ ID NO.: 1-56; (b) a nucleotide sequence encoding the polypeptide which is encoded by the nucleotide sequence recited in (a); (c) a nucleotide sequence which hybridizes to the nucleotide

- 44 -

sequence of (a) and/or (b), or to its complement, under conditions of moderate to high stringency; or (d) a fragment of any one of the nucleotide sequences of (a)-(c), which fragment retains an immunological property and/or a biological activity of the recited nucleotide sequence of (a)-(c).

5

17. A vector comprising a polynucleotide according to any of Claims 13-16.

18. A host cell containing a polynucleotide or vector according to Claims 13-17.

10 19. An attenuated bacterium containing a mutation in a gene comprising a nucleotide sequence which is capable of hybridising to any one of the nucleotide sequences defined by SEQ ID NO:1-56, under conditions of moderate to high stringency.

15 20. An attenuated bacterium according to Claim 19, having a plurality of mutations, occurring within a single gene or within different genes.

21. A composition containing a bacterium according to Claims 19 or 20.

20 22. A composition according to Claim 21, comprising a plurality of different attenuated bacteria, having different mutations in the same virulence gene and/or bacteria having similar, or different, mutations in two or more different genes.

25 23. A composition according to any one of Claims 21 or 22, further comprising one or more pharmacologically acceptable adjuvants, diluents, carriers, vehicles and/or excipients.

30 24. A composition according to any of Claims 21-23, which is packaged in a form suitable for administration by intravenous, intradermal, intramuscular, intramammary, intraperitoneal and/or subcutaneous injection, and/or by oral, sublingual, nasal, anal or vaginal delivery.

- 45 -

25. A composition according to any of Claims 21-24, which is selected from the group consisting of an immunogenic composition, a therapeutic (e.g. prophylactic) composition and a vaccine.
- 5 26. A composition according to Claim 25, wherein the composition is a vaccine whose administration to an animal confers a degree of cross-protection.
- 10 27. Use of a bacterium according to Claims 19 or 20 in the manufacture of a medicament for the therapeutic treatment or prophylactic protection of an animal against infection by the corresponding wild-type bacterium (or a different strain or serotype thereof).
- 15 28. An isolated *A. pleuropneumoniae* virulence polypeptide.
- 20 29. A method of producing a virulence polypeptide according to Claim 28, comprising the steps of: (i) culturing a host cell according to Claim 18 under conditions that permit the expression of the polypeptide; and (ii) recovering and optionally isolating the expressed polypeptide from the host cell, or from its surrounding medium.
30. A virulence polypeptide encoded by a polynucleotide according to any of Claims 13-16.
- 25 31. A composition comprising a polypeptide according to Claim 28 or 30.
32. A composition according to Claim 31, further comprising one or more pharmacologically acceptable adjuvants, diluents, carriers, vehicles and/or excipients.
- 30 33. An antibody which specifically recognizes a polynucleotide or polypeptide according to any of Claims 13-16 and Claim 28 or 30.

- 46 -

34. A method for identifying an anti-bacterial agent which is capable of modulating the function of an *A. pleuropneumoniae* virulence gene of the present invention, or of a homologous gene in a related species.
- 5
35. A method according to Claim 34, comprising screening potential agents for their ability to interfere with the expression and/or biological activity in a host bacterium of the gene products encoded by the nucleotide sequences set forth in any one of SEQ ID NO.:1-56.
- 10
36. An agent identified by the method of Claims 34 or 35.
37. A method of modulating the transcription of such virulence genes through the use of oligonucleotide-directed triplet helix formation.
- 15
38. A composition comprising an anti-bacterial agent according to Claim 36.
39. A method of treating an animal suffering from a *Pasteurellaceae* (e.g. an *A. pleuropneumoniae*) infection by administration of an anti-bacterial agent or composition according to any of Claims 36 or 38.
- 20
40. Use of an agent or composition according to Claims 36 or 38 in the manufacture of a medicament for the treatment of a *Pasteurellaceae* infection and/or porcine pleuropneumonia.
- 25